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The effects of feeding monensin on rumen microbial communities and methanogenesis in bred heifers fed in a drylot☆

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ABSTRACT

Drylot beef cow-calf systems are viable alternative management systems to traditional forage-based systems. In such confinement, adding ionophores such as monensin to the diets of beef cattle is common, and has been shown to improve feed efficiency and increase average daily gain. The addition of monensin is also commonly utilized as a strategy for methane mitigation, as this ionophore class antimicrobial acts to interfere with ion flux primarily within Gram-positive cells through its action as an ion carrier. It is widely accepted that suppression of these ruminal organisms results in the reduction of substrates for rumen methanogenic archaea, reducing methane production. However, several studies have indicated that cattle may adapt to monensin, and within weeks of feeding, may return to prior levels of methane production. Our hypothesis is that feeding monensin to bred beef heifers in confinement will temporarily decrease methane production from shifts in the methanogenic archaeal and bacterial communities. Sixteen fall-born bred heifers were randomly assigned to 2 treatment groups (n = 8 per treatment) and were fed a control diet or a diet containing monensin for 70 days using headgates. In vivo gas exchange of oxygen consumption, carbon dioxide and methane production were measured for 24-h periods throughout the trial using individual calorimeter head boxes. Rumen content sampling was conducted on day 0, 18 and 53 of the trial through oral lavage. Upon completion of sampling, DNA was isolated for ruminal bacteriome composition utilizing deep, next-generation sequencing of the V1-V3 hypervariable regions of the 16S bacterial rRNA gene. Level of methanogen 16S rRNA was quantified using qPCR. There was a significant reduction in phylum SR1 ($P < 0.05$). The abundance of several OTUs was reduced between treatment with monensin, including *Anaerofustis* ($P < 0.0001$), *Shuttleworthia* ($P < 0.0001$) and Order Bacteroidales ($P = 0.003$). No significant shifts in key ruminal methanogenic archaeal groups as a percentage of total methanogen 16S rRNA occurred ($P > 0.05$). Heifers fed monensin did not produce significantly less methane than the control ($P > 0.05$) on a liters per day basis, which was consistent throughout the study. These data suggest methane production is not reduced long-term when animals are fed monensin in confinement. Additionally, the data suggest that monensin supplementation does not suppress all classical Gram-positive populations, rather it influences finer shifts in bacterial species that may be key in ruminant function. Determining the stability of the ruminal microbiome over time in heifers fed monensin may provide further insights to long-term methane mitigation in cow-calf systems.

1. Introduction

Bovine rumen methanogenesis has been criticized as one of the most widely attributing causes of increased greenhouse gases in the atmosphere. According to a study conducted by the EPA in 2010, agriculture contributed 9% of greenhouse gas emissions, and almost one-third of those gases were methane (CH_4). Production of methane gas indicates a loss of energy from the animal (Lee et al., 2002), and can range from 2.5 to 12% of gross energy lost as methane (Russell, 2002). In an effort to combat methane production and increase feed efficiency of ruminant animals, the replacement of roughages with concentrates in the diet has proven effective (Beauchemin et al., 2008; Ellis et al., 2008). Methane

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emissions are decreased when cattle consume more highly digestible feedstuffs; consuming legumes rather than grasses and silage rather than hay (Benchaar et al., 2001). Lovett et al. (2006) determined markedly reduced methane production, by as much as 9.5% between a low and high concentrate diet, lending further support to the transition of cattle to a concentrate diet for methane mitigation. In addition to utilizing concentrate diets, the use of supplemental ionophores, primarily monensin, have decreased methane production and improved both daily gain and feed efficiency in cattle (Russell and Strobel, 1989; Grainger et al., 2008). A recent review suggesting various methane abatement strategies highlights monensin supplementation as a feasible strategy when animals are fed a high concentrate diet while maintaining adequate or increasing feed efficiency (Hook et al., 2010). As methane production represents a loss of available energy to the animal, less eructation of methane should contribute to better feed efficiency, reducing overall costs since feed represents the largest input cost associated with cattle production (Nkrumah et al., 2006).

Within the rumen microbiome, bacteria, specifically numerous Gram-positive bacteria, produce a majority of lactic acid and hydrogen (H_2) from fermentation, which can often diminish feed efficiency rates (Weimer et al., 2008). When monensin is added to the diet, Gram-positive bacteria are reduced via disruption of the ion-flux mechanism found in the lipopolysaccharide layer prokaryotic cells (Bergen and Bates, 1984; Russell and Houlihan, 2003; Hamilton et al., 2010). Hydrogen is an essential energy source for methane producing archaea (Russell, 2002), and thus disruption of the microbial-produced hydrogen due to monensin supplementation will reduce enteric methane emissions (Ellis et al., 2008). While feeding monensin in cattle diets may result in a potential decrease in methane output in short term studies where supplementation is less than six weeks, methane output in long term studies has inconclusive and often inconsistent results. This may be the result of microbial adaptation to monensin, where recent studies have suggested that the gut microbiome in cattle may adapt to monensin over time, which in turn may result in the return to prior levels of methane production.

It is critical to understand the importance of rumen methanogenesis and the role monensin plays in decreasing methane production. With limited forage availability, especially in years of extreme environmental stress such as drought, animals will often be managed more intensively in a drylot system. The aim of the present study was to determine the role of monensin on methane production from pregnant beef heifers fed in a drylot environment, and rumen microbial adaptations to the presence of monensin. We hypothesized that the addition of 150 mg of monensin to the diet of pregnant beef heifers fed a roughage diet in a drylot will produce significantly less methane and have decreased populations of Gram-positive bacteria and methanogenic archaea compared to those on the control diet.

2. Materials and methods

The U.S. Meat Animal Research Center (USMARC) Institutional Animal Care and Use Committee reviewed and approved all animal procedures. The procedures for handling cattle complied with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

2.1. Animals and experimental design

Twenty heifers born in fall of 2014 of the MARC III composite breed type were used for this study: ¼ Angus, ¼ Hereford, ¼ Pinzgauer and ¼ Red Poll. Heifers were synchronized prior to selection using a gonadotropin releasing hormone (GNRH) and prostaglandin 7-day protocol. On day 1 of this administration, heifers were administered an intramuscular injection of 100 micrograms of GNRH. Following injection, estrotect patches (Genex Cooperative Inc.) were placed on the tailhead of the heifers. Four days later on day 5, two bulls were placed with heifers for natural service. On day 7, heifers received an intramuscular injection of PGF2α and estrotect patches were read. Bulls remained with the heifers for 30 days and pregnancy was detected via transrectal ultrasonography approximately 30 days later.

The resultant sixteen 2014 fall-born heifers with confirmed pregnancy were sorted into two treatment groups, one fed a control diet and one fed a diet containing monensin. Animals were fed the diet over 53 days. Once the animals were separated into the treatment groups, the heifers were fed at a rate of 0.138 megacalories of metabolizable energy per kilogram of metabolic body size. Individual feed intake was measured using Calan Broadbent head gates (American Calan Inc.) Diets consisted of 80% corn stalks, 10% corn silage, 7% wet distillers grains with solubles, and either a vitamin and mineral supplement with or without 150 mg of monensin per day, respectively. Specifically, diets consisted of 71.89% dry matter, 83.19% organic matter, 66.81% neutral detergent fiber, 40.78% acid detergent fiber, and 8.63% crude protein based on laboratory analyses (Servi-Tech Labs, Hastings, NE). The corn stalks were ground prior to feeding (approximately 7.62 cm chop length).

2.2. Rumen content sampling

On days 0, 18 and 53 of monensin feeding, rumen samples were collected via aspiration from the rumen using a gastric tube. Ruminal content was collected the morning of days 0, 18 and 53, and was transferred to 50-mL conical tubes and frozen at −80 °C for long-term storage until processing.

2.3. Quantification of gaseous emissions

Prior to each rumen content collection period, eight animals (4 from each treatment) were placed in metabolism stanchions were gas exchange and nutrient balance were conducted. The following week, the remaining eight animals were moved to metabolism stanchions where gas exchange measurements were conducted. This procedure has been previously performed by Hales et al. (2014). At least 3 air turnovers were allowed before the gas measurements were determined. Animals were allowed their daily dietary allotment prior to beginning of gas collections, and >85% of the diet was typically consumed. Gas exchange was determined by pulling air through the box across a temperature-compensated dry test meter to determine airflow leaving the box. Real time air temperature and humidity were determined. Proportional samples of background air entering the box and air exhausted from the box were collected in polyethylene-aluminum-Mylar laminate gas bags to form a composite air sample for the collection period for each individual box. Gas samples were analyzed for CO_2, and CH_4 according to Nienaber and Maddy (1985), and specifically CH_4 was analyzed using an infrared gas analysis system (AR-60A, Anarad, Inc., Santa Barbara, CA). Before gas measurements were collected, each head box was calibrated for O_2 consumed and CO_2 produced by burning absolute ethanol with alcohol lamps. Recoveries ranged from 98% to 101% in all head boxes

2.4. DNA Extraction, Amplification, sequencing

Deoxyribonucleic acid was extracted from rumen content samples using a repeated bead beating plus column method (Yu and Morrison, 2004). Following cell lysis, 10 M ammonium acetate (260 μL) was used to precipitate and remove the impurities followed by equal volume isopropanol precipitation for the recovery of the nucleic acids. Supernatants were treated with 2 μL ribonuclease (10 mg/mL) and proteinase K (QIAamp DNA Stool Mini Kit) followed by the use of QIAamp columns from the Qiagen DNA Stool Mini Kit (Qiagen, Hilden, Germany). Metagenomic DNA concentration was determined using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE).

Amplicon library preparation was performed using polymerase chain reaction (PCR) for 30 cycles at an annealing temperature of 58°C, targeting the V1-V3 hypervariable regions of the bacterial 16S rRNA gene. Modified universal primers 27F(5′-Adapter/Index/ AGAGTTTGA TCCTGGCTCAG) and 519R (5′-Adapter/ Index/ GTATTACCGCGGCTG CTG) including TruSeq indices and adapters were used with AccuPrime Taq high fidelity DNA Polymerase (Life Technologies, Carlsbad, CA) to produce the sequencing libraries. Products were quality checked with gel electrophoresis. Libraries were then purified using AmPure beads (Agencourt, Beverly, MA) and quantified using a Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE) and by real-time PCR on the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany). The PCR amplicon libraries were sequenced using the 2 × 300, v3 600-cycle kit and the Illumina MiSeq sequencing platform (Illumina, Inc., San Diego, CA) at the United States Meat Animal Research Center (US MARC; Clay Center, Nebraska).

2.5. DNA sequencing analysis

Amplicon sequence reads processed using the Quantitative Insights Into Microbial Ecology (QIIME) bioinformatics pipeline, version 1.9.1 (Caporaso et al., 2010) and Mothur version 1.36.1 (Schloss et al., 2009). Sequences were quality trimmed on the Galaxy server and those with quality score ≥30 were retained. Read lengths shorter than 300 bp were removed and adapters/index sequences were trimmed. Chimeric sequences were identified and subsequently removed using usearch61 (Edgar, 2010), and sequences classified as chloroplasts and mitochondria were removed. Samples were subsampled to a depth of 25,000 sequences. The resulting subsamples were clustered using the UCLUST module from QIIME into operational taxonomic units (OTU) with a pairwise identity threshold of 97% (Caporaso et al., 2010) and assigned to taxonomy using UCLUST and the Greengenes v13_8 16S rRNA database as a reference, and pooled into taxa for taxonomic analysis. Alpha-diversity was analyzed using observed species, chao1, Shannon's Diversity Index and Faith's Phylogenetic Diversity. For analysis, only OTUs present in ≥50% of samples were used.

2.6. Quantification of methanogen 16S rRNA

Following DNA extraction, real-time quantitative polymerase chain reaction (qPCR) was performed to determine level of methanogen 16s rRNA. This was performed similarly to the established method by Freetly et al. (2015). Oligonucleotide primers used for qPCR analyses targeted eight methanogen groups: Methanomicrobiales (order), Methanobacteriales (order), *Methanosarcina* (genus), *Methanobacterium* (genus), *Methanobrevibacter ruminantium* + *Mbb. cuticularis*, and *Methanobrevibacter smithii* + *Mbb. wolinii* + *Mbb. thaueri* + *Mbb. gottschalkii* + *Mbb. woesei* as described in Freetly et al. (2015). Standard curves for each primer set were produced in order to quantify copy numbers in each sample. PCR amplicons were cloned into the Topo vector and transformed into One Shot TOP10 competent cells (Topo TA cloning kit, Invitrogen, Carlsbad, CA). These clones were used to generate reference plasmids for quantifying total bacteria and total methanogenic archaea 16S rRNA genes. DNA from the transformed *E. coli* cells was obtained by using the QIAprep spin miniprep kit (Qiagen) and plasmid purity was confirmed by agarose gel electrophoresis. Concentrations of DNA were determined with a Nanodrop 1000 (Thermo Scientific, Wilmington, DE) and gene copy numbers were calculated with the Thermo Scientific DNA copy number calculator tool (http://www.thermoscientificbio.com/webtools/copynumber/). Samples utilized as plasmid controls were diluted to 10^2 through 10^8 copies of plasmid copies/μL. Calculations of copy numbers for each sample were performed using standard curves calculated by Bio-Rad CFX Manager software (version 3.1)

2.7. Statistical analysis

Bacterial alpha-diversity, methane and carbon dioxide gas emissions, as well as methanogens from days 0, 18 and 53 of supplementation, were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc. Cary, NC). Days of treatment were analyzed separately and then compared as a whole. The model included Gram-positive, Gram-negative bacteria, quantities of methane and carbon dioxide emitted, quantities of methanogens, and treatment of monensin or control. All data were checked for normality prior to analysis using PROC UNIVARIATE. Subsequently, methane emission data were normalized using a log transformation prior to analysis. Bacterial OTU were statistically analyzed using the Kruskal-Wallis test. Data were considered significant at a P-value < 0.05, with tendencies declared at P-values between 0.05 and 0.10.

3. Results

Collectively, the sampled rumen contents of the 16 heifers resulted in a total of 1,200,000 overall reads after quality control and chimera detection and removal. Individual samples generated an average of 51,719 cleaned sequence reads, ranging from 25,733 to 190,044 sequences. Within the total cleaned sequences, an average of 2744 ± 162 OTU were detected per sample. The alpha diversity of the operational taxonomic units (OTU) revealed no difference between treatments in Shannon's Diversity Index, observed OTUs, or chao1 metrics (Table 1). However, there was a tendency at Day 18 for a reduction in Faith's Phylogenetic Diversity with the group fed supplemental monensin.

There was no effect of treatment on gram-positive or gram-negative relative abundances throughout each of the days tested ($P > 0.05$, Fig. 1). However, 14 specific taxa were identified to be significantly altered when animals were fed the monensin compared to the control diet ($P < 0.05$, Table 2). Among those 14, 9 taxa were significantly reduced at Day 18 of supplementation, with only 5 taxa significantly reduced at day 53. An additional 9 taxa tended to be altered in monensin-fed animals ($0.05 < P < 0.1$, Supplementary Table 1). While not statistically significant, it is noteworthy that a numerical reduction of overall Gram-positive bacteria amounts occurred at both days 18 and

Table 1
Diversity statistics between treatments by day.

Treatment[1]	Day	No. Observed OTU[2]	Chao1	Shannon's Diversity	Faith's Phylogenetic Diversity
Control	0	3170 ± 131	9694 ± 583	9.24 ± 0.13	114.21 ± 4.50
Monensin	0	3012 ± 197	9338 ± 1262	9.10 ± 0.23	111.00 ± 5.78
Control	18	2744 ± 123	10,512 ± 1183	9.08 ± 0.12	118.96 ± 4.99[a]
Monensin	18	2693 ± 170	10,075 ± 986	9.00 ± 0.25	114.51 ± 4.21[b]
Control	53	2507 ± 196	7354 ± 735	8.61 ± 0.26	94.78 ± 4.15
Monensin	53	2518 ± 154	7634 ± 614	8.66 ± 0.19	94.29 ± 3.78

[1] $n = 8$.
[2] OTU: Operational Taxonomic Unit.
[a,b] Means within column by day with tendencies to differ at $0.05 < P < 0.1$.

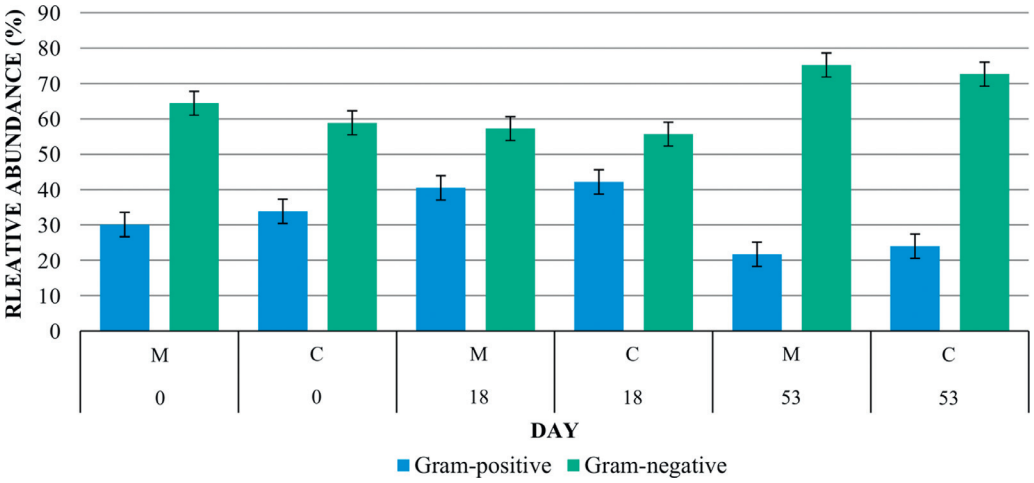

Fig. 1. Relative abundance of Gram-positive and Gram-negative bacteria by day in bred heifers fed a diet with and without monensin. Gram-reaction composition was compared based on relative abundance (sequences of a taxon/total sequences in a sample) Relative abundances did not differ ($P > 0.05$). Error bars represent SEM.

53 between treatments. When comparing the ratio of Firmicutes-to-Bacteriodetes across sampling days, there was no effect of treatment ($P > 0.05$, Supplementary Fig. 1) throughout the study.

There was no effect of treatment with monensin on methane or carbon dioxide gas emissions ($P > 0.05$, Fig. 2) when reported as total liters per heifer. Overall, there was no effect of treatment on methanogenic archaea quantities from ruminal content ($P > 0.05$, Fig. 3). There was a tendency for Methanomicrobiales abundance to be reduced due to treatment ($P = 0.0684$, Fig. 3), but there was no interaction of treatment by day. *Methanobacterium* was reduced by day ($P = 0.0004$) but not by treatment (Fig. 3). Additionally, the groups including the genera *M. smithii* + *Mbb. wolinii* + *Mbb. thaueri* + *Mbb. gottschalkii* + *Mbb. woesei* and *M. ruminantium* + *Mbb. cuticularis* were both reduced between days 0 and 18, and 0 and 53 (Fig. 3), but were unaffected by treatment of monensin.

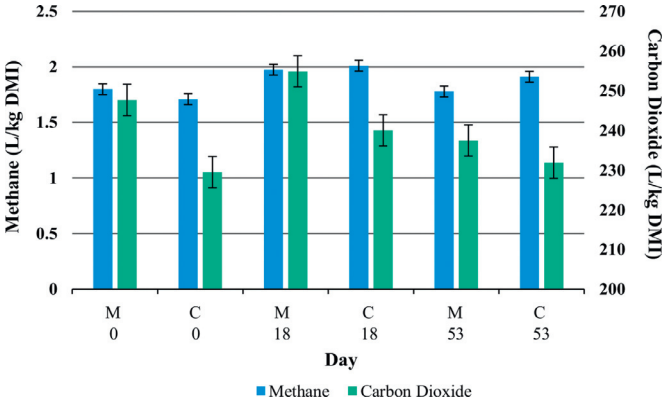

Fig. 2. Methane and carbon dioxide emissions by day in bred heifers fed with and without monensin. Total emissions of both methane and carbon dioxide did not differ by day nor by treatments ($P > 0.05$). Error bars represent SEM.

4. Discussion

Previous studies have determined the efficacy of monensin as a short term (less than six weeks) supplement while the efficacy of long term supplementation (greater than six weeks) is diminished, suggesting adaptation from microbial populations (Guan et al., 2006; Weimer et al., 2008). Many studies have provided conflicting results with the use of monensin. In a study conducted on lactating dairy cattle in 2007, animals who received monensin supplementation in the diet for 6 months experienced a sustained 9% decrease in methane emissions (Odongo et al., 2007), and no adaptation from rumen methanogenic microbes was determined. However, no differences in this study were noted until the fourth month of the study. When lactating dairy cattle were fed an alfalfa based ration, Hamilton et al. (2010) determined that monensin had no effect on reducing methane emissions nor any changes in the colonic microbial communities at both day 14 and day 60 of monensin supplementation. Although the present study sampled ruminal content, no significant bacterial or archaeal population changes were observed, similar to the results from Hamilton et al. (2010). Similarly to data presented by Weimer et al. (2008), the effects of monensin in the diet of the present

Table 2
Significant gram-positive and gram-negative bacteria by day.

Classification	Gram-reaction	Day	Control (%)[a]	Monensin (%)[a]	*P*-value[b]	SEM
Anaerofustis	Positive	18	0.021	0.003	<0.001	0.003
Shuttleworthia	Positive	18	0.009	0.084	<0.001	0.012
Order Bacteroidales	Negative	18	20.781	17.08	0.003	0.703
Order RF39	Positive	18	1.319	0.728	0.006	0.116
Family Synergistaceae	Negative	18	0.011	0.027	0.02	0.004
Anaerovibrio	Positive	18	0.006	0.015	0.024	0.002
Prevotella	Negative	18	15.932	23.279	0.024	1.69
Phylum SR1	Negative	18	0.104	0.034	0.025	0.016
Treponema	Negative	18	0.431	0.336	0.027	0.022
Family Veillonellaceae	Positive	18	0.036	0.059	0.031	0.006
Family S24-7	Negative	53	4.11	2.725	0.009	0.285
Streptococcus	Positive	53	0.014	0.007	0.026	0.002
Order Clostridiales	Positive	53	0.269	0.158	0.035	0.026
SHD-231	Negative	53	0.158	0.114	0.038	0.011

[a] Represented as relative abundance.
[b] Differences are significant at $P < 0.05$.

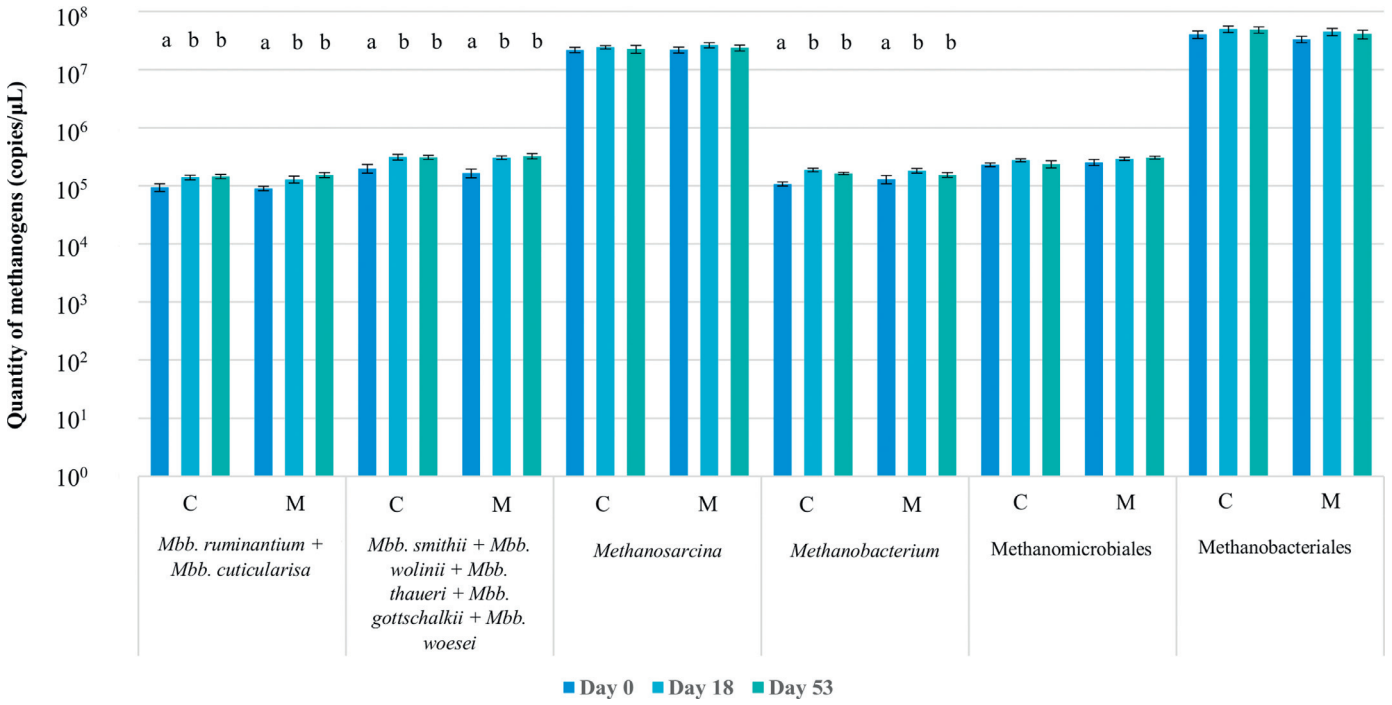

Fig. 3. Quantity of methanogens from rumen content of bred heifers fed with and without monensin. Data was determined by real-time quantitative PCR. Error bars represent SEM. Subscripts within archaeal group indicate taxa significantly reduced between days ($P < 0.05$).

study did not contribute to an overall suppression of ruminal Gram-positive bacteria. However, as fourteen bacterial species and four archaeal communities shifted throughout the current study, this may indicate the effects of monensin on the rumen microbial communities are still relatively unknown or interact with key species to influence the ruminal environment. Abrar et al. (2015) experienced similar findings where methanogen numbers nor Gram-positive bacterial amounts were affected by the inclusion of monensin in the diet (2015). Future studies on specific bacterial populations can lead to further understanding of the complex mechanism of monensin on the rumen microbiome.

It is well documented that when more readily digestible feedstuffs (legumes compared to grasses, and silage compared to hay) are fed to ruminants, methane emissions are reduced (Paterson et al., 1994; Beauchemin et al., 2009; Hales et al., 2015), lending support to include these feedstuffs in greater amounts in diets. Additionally, when unsaturated fats and oils are supplemented, methanogens are reduced and methane production is decreased (Patra, 2013; Tomkins et al., 2015; Castillo-González et al., 2016). When dairy cattle were fed a 40:60 forage to concentrate diet, Castillo-Gonzalez et al. found that monensin supplementation caused a 7% decrease in methane emissions, and supplementation of monensin with tallow in the diet caused a large decrease in Gram-positive bacteria in the rumen that would contribute to methane production (2016). While the use of concentrate diets has previously been identified to possibly reduce methane emissions by as much as 27% (Guan et al., 2006), no methane abatement occurred in the present study which utilized roughages for the majority of the diet. Similarly, when Agle et al. (2010) provided dairy cows a low or high concentrate diet, no methane reduction was observed in the treatments compared to those fed on a control diet. The variation with use of monensin and its effects on methane reduction could indicate higher dosages are needed to provide more consistent results. The present study supplemented monensin at only 150 mg per heifer per day in this roughage-based diet, dry-matter basis, and no methane reduction was reported, whereas Odongo et al., (2007) reported a dose of monensin at 24 mg/kg DM was sufficient for methane reduction, when animals were receiving a more concentrate diet.. Although Ranga Niroshan Appuhamy et al. (2013) observed that beef steers receiving 379 g/kg DM provided a significant reduction in methane emission, no exact dosing of monensin has been reported for consistent, long-term methane abatement across sex (steer, heifer, bull or cow) or production use (beef or dairy).

Specific rumen bacterial communities have been highlighted among high and low methane producing animals; specifically those who produced less methane had greater amounts of *Fibrobacter* spp., and *Prevotella byrantii*, while animals who had higher methane emissions had increased amounts of *Clostridiales* spp., Bacteriodaetes, *Alphaproteobacteria* and *Ruminococcus* taxa (Tapio et al., 2017). In the present study, the several taxa that were significantly reduced or tended to be reduced are associated with those previously identified by Tapio et al. (2017) as part of the taxa among high methane producing animals. With only five taxa significantly altered at day 53, this can potentially provide evidence that an adaptation of finer changes in the microbial communities exists. This suggests that while large shifts in the rumen bacteriome and methane production were not determined, finer changes within the rumen may indicate strategies for methane abatement.

Despite previous studies that have used steers as the animal model to establish methane mitigation methods (Thornton and Owens, 1981; McCaughey et al., 1997; Freetly et al., 2015), the use of steers with a typical 15-month life-span reduces the potential for long-term methane reductions in the industry. Steers contribute to the majority of beef production in the United States, which has increased 3% since 2015 to 14.5 million head (Fereira, 2017), however, replacement heifers have greater longevity than market steers, and thus contribute to a greater proportion of the methane production attributed to beef cattle. Further studies using replacement heifers intended for cow-calf production systems and the use of monensin can be utilized to determine long term methane reduction strategies.

5. Conclusion

Based on the results of this study, monensin supplementation at 150 mg per animal per day in the diet fed to bred beef heifers in a drylot may not be an effective measure to reduce long-term methane

production. While fourteen unique bacterial species and four methanogenic archaea were identified differing between heifers consuming monensin and those on the control diet, overall shifts among Gram-positive, Gram-negative and methanogenic archaeal communities were not observed in this study. Nonetheless, these finer microbial changes may begin to elucidate further ways to include monensin in the diet for methane reduction and increased feed efficiency. As the most numerous shifts in Gram-positive and Gram-negative organisms occurred at day 18, feeding a moderate dose of monensin for a short period of time may have greater use to understanding the complex effects of monensin on the rumen microbial communities. Further understanding of these finer microbial shifts may result in improved use of monensin for increasing feed efficiency, reducing production costs and reducing methane production.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

EAM assisted in coordinating the research project, performed DNA sequencing analyses of bacteria and methanogenic archaea, analyzed data, and wrote the manuscript. KEH conceived the research, designed and planned the experiment, conducted sampling and physiological data analysis. ALP conducted qPCR of methanogenic archaea. HCF designed and planned the experiment. JEW designed and conducted qPCR of methanogenic archaea and assisted in coordinating the research project. CNH conducted sampling and aided in physiological data analysis. TAW designed and planned the experiment. JES designed and planned the experiment. PRM conceived the research, designed and planned the experiment, coordinated the research project, and assisted in writing the manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2018.03.019.

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